REMARKS

Claims 134, 139-143, 145, 148-155 and 157-176 are pending. Favorable reconsideration is respectfully requested.

The present invention relates to a vaccinating composition against a *Plasmodium* parasite which is infectious in man. This vaccine composition comprises as an active principle a recombinant protein whose polypeptide sequence comprises a 19 kilodalton (p19) C-terminal fragment of a surface protein 1 of a merozoite form (MSP-1 protein) of a *Plasmodium* parasite that is infectious in man, other than *Plasmodium* vivax. This C-terminal MSP-1 fragment induces an immune response and can inhibit parasitemia in vivo in a host infected with said *Plasmodium* parasite, as well as remaining anchored to the surface of said *Plasmodium* parasite at an end of its penetration phase into human erythrocytes during an infectious cycle. This recombinant protein comprises conformational epitopes, which are contained in two epidermal growth factor regions and is unstable in a reducing agent, wherein said 19 kilodalton (p19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) has atomic coordinates in Annexes I or III; and NMR fingerprints of Figures 12.0a to 12.0c or 12.2a to 12.2c. This vaccinating composition contains alum as an adjuvant.

The present invention also relates to recombinant proteins, and oligomers of these recombinant proteins.

The recombinant proteins produced in the present invention form a conformationally correct analog of the native structure which is of primary importance for the protective efficacy which has been demonstrated in several primate models. These primate models are known to be the best animal models in interpreting the efficacy of a malaria vaccine in humans. Furthermore, it is the first time that an alum formulation of a C-terminal MSP1 antigen has shown significant protection against malaria in any primate model. This

observation is particularly important since alum is the only adjuvant accepted for use in humans.

This has been documented in Miller et al (1997), a copy of which is enclosed.

It will be demonstrated below that none of the cited prior art in which a C-terminal MSP-1 antigen has been produced in *E. coli* retains the structural conformation in such a manner that it can be used efficiently as a vaccine to provide protection against malaria in man.

The rejection of Claims 153, 159, 162, 165, 169, 172 and 175 under 35 U.S.C. §103(a) over Longacre (1995) in view of Longacre et al (1994) is respectfully traversed. The cited references fail to suggest the claimed invention.

Longacre (1995) deals exclusively with the description of the gene sequence for P. cynomolgi C-terminal MSP-1p42. There is no indication or demonstration in this paper that this sequence can be used to generate recombinant proteins in any expression system, nor whether such putative antigens generated would induce an immune response which can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite.

Longacre (1995) fails to disclose or even suggest a recombinant protein which is the C-terminal fragment of a MSP-1 protein of SEQ ID NO:1 from Asn at amino acid position 3 to Ser at amino acid position 95 (Claim 151) or SEQ ID NO: 4 from Asn at amino acid position 3 to Ile at amino acid position 116 (Claim 152), which fragment induces an immune response which can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite.

Furthermore, Longacre (1995) does not suggest that upstream of the 19 kilodalton C-terminal fragment the C-terminal end of p33 of a MSP1 protein of a *Plasmodium* parasite can be inserted (Claim 176).

Longacre et al (1994) describes recombinant proteins from *Plasmodium* vivax MSP-1 produced in a baculovirus expression system. The *Plasmodium* vivax proteins were detected in cellular lysates (fig. 3 and 4). This reference also discloses that these recombinants proteins reacted with hyperimmune human antisera on immunoblots. Longacre et al does not disclose a recombinant protein that can induce an immune response which can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite.

The combination of these references fails to render the presently claimed invention obvious since there is no showing of inhibition of parasitemia in vivo in a host infected with a *Plasmodium* parasite. Without such a showing, Applicants submit that the mere hypothetical suggestion to the skilled artisan of a vaccine in both these references without a true experimental showing would lead the skilled artisan to conclude that there would be no expectation of success that parasitemia could be inhibited in vivo in a host infected with a *Plasmodium* parasite. In this regard, the Examiner is referred to paragraph 4 of the Longacre Declaration

Therefore in view of the above, withdrawal of this rejection is respectfully requested.

The rejection of Claims 134, 139 to141, 143, 145 and 148 to 150 under 35 U.S.C. §103(a) over Longacre (1995) in view of Longacre et al (1994) and further in view of Holder et al. (U.S. 5,720,959) is respectfully traversed. The cited references fail to suggest the claimed invention.

Claim 134 is directed to a vaccinating composition against a *Plasmodium* parasite which is infectious in man. This vaccinating composition comprises as an active principle a recombinant protein comprising a 19 kilodalton C-terminal fragment of MSP-1 protein of a *Plasmodium* parasite, other than *Plasmodium* vivax, which induces an immune response and can inhibit parasitemia in vivo in a host infected with the *Plasmodium* parasite.

Longacre (1995) concerns the sequencing and comparison of *Plasmodium* cynomolgi C-terminal MSP1 with two *Plasmodium* vivax variants. There are no immune response experiments set forth in this reference either in vivo or in vitro concerning the inhibition of parasitemia.

Longacre et al (1994) discloses *Plasmodium* vivax MSP-1 recombinant proteins. *Plasmodium* vivax is excluded from claim 134 and therefore this reference should be considered irrelevant.

Holder et al, as a whole, disclose a recombinant fusion protein comprising both EGF-like domains of *Plasmodium* yoelii MSP-1 fused to the C-terminus of glutathione S-transferase, expressed in bacterial cells (E.coli). *Plasmodium* yoelii is a rodent parasite that is not infectious in man.

The examples in Holder et al illustrate that only Freund's complete and Freund's incomplete were used as adjuvants. There was no example using the EGF-like domains and alum as an adjuvant in the mouse model to determine whether parasitemia could be prevented after challenge.

Indeed, as set forth at paragraph 5 in the Longacre Declaration, it was well known in the immunological art that the efficacy of recombinant protein subunit vaccines is generally highly adjuvant dependent. The mere mention of the possibility of using alum as an adjuvant in a malaria vaccine would be mere hypothesis without experimental results that alum could in fact work. The use of adjuvants in the malaria vaccine art is thus unpredictable. Without experimentally demonstrating that alum can be successfully used, the mere mention of this adjuvant would not be sufficient for the skilled artisan to combine it with an MSP-1 antigen, especially since alum was known as a weak adjuvant (see, Miller et al 1997).

Therefore, without demonstrating that alum could in fact work in animal models with the MSP-1 C-terminal recombinant MSP-1, Applicants submit that the skilled artisan would have no expectation of success of a vaccine for malaria containing alum as an adjuvant with an MSP1-19 antigen.

Moreover, as set forth in the specification, it was only the present invention that first demonstrated that significant protection of parasitemia was achieved using alum (see also, paragraph 5 of the Declaration).

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

The rejection of the claims under 35 U.S.C. §103(a) over the combined teachings of Chappel and Holder, Miller et al, Longacre et al (1994) and Longacre (1995) is respectfully traversed. Those references fail to suggest the claimed invention.

Chappel and Holder disclose monoclonal antibodies that inhibit *Plasmodium* falciparum invasion in in vitro culture and recognize the first growth factor (EGF-1) domain of MSP-1. Several recombinant proteins were constructed using the EGF-1 and EGF-2 domains as fusion proteins with glutathione S-transferase. Several monoclonal antibodies were used to test whether they bind to the recombinantly produced proteins. Five antibodies recognized the first EGF-like domain and therefore this domain is a target for antibodies capable of inhibiting parasite invasion in vitro.

There is no experimental evidence in Chappel and Holder indicating that the recombinant proteins can induce an immune response which can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite.

Furthermore, it was concluded at paragraph 6 of the enclosed Declaration that relatively small proportions, if any, of the recombinantly produced bacterial products retain their native confirmation. This conclusion was in fact substantiated by the publication of Burghaus et al in Annex II of the Declaration and at paragraph 7. This publication discloses

that when Aotus nancymai monkeys were immunized with almost the same fusion proteins as that described in Chappel and Holder, using alum as an adjuvant, there was no protective immunity and the monkeys became parasitemic, i.e., showed no significantly reduced parasitemia as compared to the control.

Moreover, Chappel and Holder disclose in the last paragraph, first column at page 308, that a single amino acid substitution in the EGF-1 binding domain at position 14 (1644 of Miller et al; glutamine to glutamate in Figure 5) strongly influenced the antibody binding of polyclonal antibodies raised to the native MSP1 protein as shown in the bottom panel of Figure 2.

Thus, a person skilled in the art would not modify glutamine to glutamate at this position.

However, independent Claims 151 and 152 refer to the 19 kilodalton C-terminal fragment of a MSP-1 protein of *Plasmodium* falciparum from ASN at position 3 to SER at position 95 of SEQ ID NO:1 (claim 151), while claim 152 recites for the MSP-1 protein of *Plasmodium* falciparum from a sequence from ASN at position 8 to Ile at position 116 of SEQ ID NO.:4. Both of these sequences contain a glutamate at position 14, and not a glutamine.

Therefore, Applicants submit that Chappel and Holder would teach away from the claimed invention.

The secondary sequence of Miller et al was only referred to in the present rejection to orient the sequences described in Chappel and Holder.

Longacre (1995) and Longacre et al (1994) have nothing to do with the MSP-1 sequences from *Plasmodium* falciparum. As discussed above, there is no demonstration in these references that show that their particular recombinant constructs can induce an immune response which can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite.

The combination of these references fails to render the presently claimed invention obvious, since the primary reference of Chappel and Holder teach away from modifying the amino acids at position 14 of the MSP-1 protein and especially not from glutamine to glutamate. Thus, the skilled artisan would not attempt to modify the sequence in this matter, which is currently claimed.

As stated in McGinley v. Franklin Sports, Inc. 262 F3d 1339, 60 USPQ2d 1001 (Fred. Cir. 2001):

> We have noted ... as "useful general rule", that references that teach away cannot serve to create a prima facie case of obviousness.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

The rejection of Claims 134, 139 to 143, 148, and 150 under 35 U.S.C. §103(a) over Chappel and Holder, Miller et al and Longacre (1995) in view of Longacre et al (1994) and further in view of Holder et al. is respectfully traversed. Those references fail to suggest the claimed invention.

Chappel and Holder fail to disclose that their anchored recombinant MSP-1 protein can induce an immune response and can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite. Indeed as evidenced in the Longacre Declaration, when a very similar *E. coli* produced antigen was administered to Aotus nancymai monkeys using alum as an adjuvant the monkeys became parasitemic, i.e., showed no significantly reduced parasitemia as compared to the control.

The secondary references were discussed above and the same arguments apply in this rejection and are therefore incorporated herein by reference. It should be recalled that Holder et al fail to demonstrate an immune response using alum. Furthermore, is was known at the filing date of the present invention that adjuvants effect the efficacy of recombinant MSP-1 vaccines, as well as the fact that alum was a poor adjuvant. Therefore, one skilled in the art

in the area of malaria vaccines would not conclude that there would be any expectation of success that alum could be successfully used with a recombinant MSP-1 protein and induce an immune response such that parasitemia can be inhibited in vivo in a host infected with a *Plasmodium* parasite.

Lacking any expectation of success by the skilled artisan this combination of references would not be undertaken. Therefore, in view of the above, withdrawal of this rejection is therefore respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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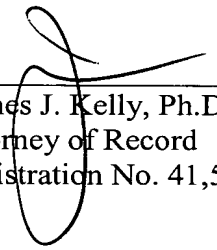

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